

ORIGINAL CONTRIBUTION

A Note on Urine Trans, Trans Muconic Acid Level Among a Sample of Thai Police: Implication for an Occupational Health Issue

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Benzene exposure is of particular concern because recent research indicating that benzene exposure can result in chronic toxicity, therefore, monitoring for benzene exposure among at-risk workers is recommended. In exposure- and risk-evaluation, the monitoring of benzene by peripheral biomarker has several advantages over technical assessment of exposure. For this purpose, the urine trans, trans muconic acid (ttMA) level is accepted as a useful monitoring tool for early diagnosis of dangerous exposure. Apart from the industrial workers, there are other occupations with high risk for benzene exposure. In this study, we study another at-risk occupation, the police. Thirty-nine urine samples were obtained from 39 Thai police working close to traffic in an urban area. All 39 samples were analyzed for ttMA level and compared to 10 other controls. The average urine ttMA level for the control and exposed group were < 0.05 mg/gCr and was 0.79 ± 1.43 mg/gCr, respectively. Significant higher urine ttMA acid level among the police were observed ($p < .05$). Working in the air pollution in the urban area can be health hazard for the police. Exposure to the benzene from automobile exhaust can be an important occupational problem for these police.

INTRODUCTION

Benzene is of particular concern because recent research indicating that benzene exposure can result in chronic toxicity [1]. At present, work with benzene is subject to the Control of Substances Hazardous to Health (COSHH)^e Regulations 1999. Hence, benzene exposure is of particular concern because of

ongoing exposure to thousands of workers in the industrial plants. Monitoring for benzene exposure among the at-risk workers is recommended and of several biomarkers, urine trans,trans-muconic acid (ttMA) determination is a helpful test for monitoring. Presently, urine ttMA is a good indicator of exposure level to benzene [2].

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^eAbbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; COSHH, control of substances hazardous to health; ttMA, trans, trans muconic acid.

In developing countries, awareness of the public health impact of exposure to volatile solvents is growing, although few of these countries have introduced policies and regulations that combat the problem effectively. Owing to the recent industrialization of Thailand, a developing country in Southeast Asia, many industrial workers are now at-risk of exposure to benzene. There have been continuous reports [3-4] about monitoring benzene exposure among these at-risk industrial workers in Thailand. However, apart from the industrial workers, there are other occupations with high risk for benzene exposure.

In this study, we study another at-risk occupation, the police. This pilot study aimed to determine the urine ttMA level among a sample of Thai police working close to traffic in an urban area. We also compared the detected level among our subjects to the previous reports in Thailand.

MATERIALS AND METHODS

Subjects

Thirty-nine healthy volunteer male police were included in this study. These police who had to work daily as traffic police in an urban area, namely Pathumwon District, Bangkok. We also performed additional investigation in 10 healthy male subjects, a group of students

studying at Chulalongkorn University, to serve as the control. These students lived in the same area, Pathumwan District, but in the dormitory about 0.5 kilometers away from roads. All subjects in this study presented the same eating and drinking habit. All subjects were healthy adults. Before the study, all were interviewed for possible exposure to benzene, especially for smoking and volatile substance abuse. The exclusion was set in a case with history of possible exposure to benzene. Conclusively, 49 subjects overall were included into this study. The sample size in this study was derived by the calculation based on our previous study.

All subjects gave informed consent. The Faculty of Medicine and Faculty of Allied Health Science, Chulalongkorn University, approved the study. Each subject provided a urine sample for laboratory analysis.

Laboratory analysis

A. Analysis for the urine ttMA level

All collected samples in this study were sent to the laboratory for further analysis for ttMA level. Determination of ttMA level was performed using high-performance liquid chromatography method as described in our recent previous study [5].

Briefly, 0.5 ml of urine sample was mixed with 2 ml of Tris Buffer containing vanillic acid as internal standard (IS).

Table 1. Urine ttMA level among police and controls.

| Group | Number of subjects | Urine ttMA (mg/gCr) | | | | |
|-----------------------|--------------------|---------------------|------------------|------------------|------------------|------------------|
| | | Mean | SD | Maximum | Minimum | 95% CI |
| Police | 39 | 0.79 | 1.43 | N/A ^a | 1.86 | 0.56-1.02 |
| Controls ^b | 10 | N/A ^a | N/A ^a | N/A ^a | N/A ^a | N/A ^a |

^a The ttMA level was beneath the limit of detection. ^b All subjects in the control group presented the urine ttMA level that lower than the detection limit of the test, therefore, the reported average ttMA level in control group was <0.05 mg/gCr.

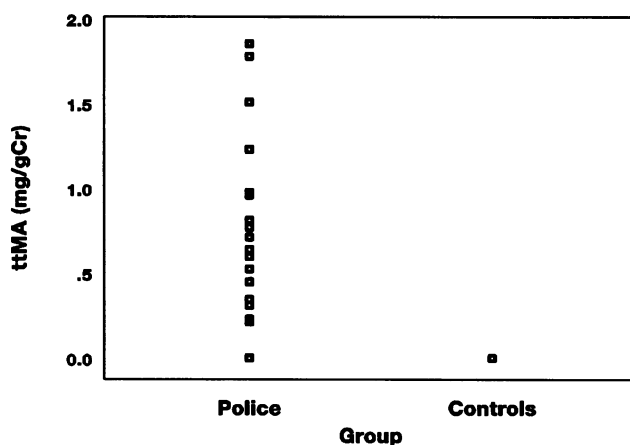


Figure 1. The data for ttMA among the police and controls.

Then this mixture was percolated through a preconditioned ion-exchange column. After rinsing the column with phosphoric acid solution, acetate buffer, and deionized water, we eluated the analytes with 2 ml of an equivolume solution of 1.5 M NaCl methanol. Of this, 100 microliter is injected into the HPLC column. The mobile phase used consisted of, per liter, 10 ml of acetic acid, 100 ml of methanol, and, the rest, 5 mM sodium acetate.

The positive and negative controls for the analysis are ttMA solution and distilled water. The flow rate was started at 1.2 ml/min. The ttMA and IS were detected at 5.5 to 6.1 and 15.1 to 16.7 minutes, respectively. Concerning the linearity study, the calibration curve proved linear with a correlation coefficient of 0.99. The lowest detection limit (sensitivity) was 0.05 mg/l. The recovery range from 91.12 to 93.36 per cent. Precision study yielded a variation coefficient of 13.6 per cent. However, stability test to assess the possible interference of storage time showed that the detected ttMA level decreased chronologically; therefore, the fresh sample is required [5]. Results are expressed as mg ttMA per gram urine creatinine (mg/gCr).

B. Detection of environmental benzene levels

In addition, the authors had a reference laboratory (Life and Environment Company, Bangkok) to perform air sampling and analysis. The environmental benzene level in both police group and controls were studied. For each group, an area sample from the ground were collected using Mini Pump SKC 224 PCX R8 with filter (air pumping rate = 0.2 l/min) during a four-hour period, 12:00 to 16:00 when there were traffic jams but no rain. Environmental benzene levels of the subjects were detected at the reference laboratory according to the standard method (Gravimetric Method: NIOSH 0500).

C. Statistical analysis

Data from all laboratory examinations in this study were systematically comprehensive collected for further statistical analysis. The statistical analysis of the results was carried by SPSS 7.0 for Windows Program. For comparison of the value of ttMA acid, the unpaired T-Test was used. Statistical significant difference was accepted at p value < .05 similar to the other medical studies.

RESULTS

A total of 39 police (all males) were included in this study. The average (mean \pm SD) urine ttMA level in these policeman was 0.79 ± 1.43 mg/gCr. The urine ttMA level in all control subjects (university students) was lower than the detection limit of the test, therefore, the reported average ttMA level in control group was <0.05 mg/gCr (Figure 1). Hence, a significant higher urine ttMA level among the policeman was observed ($p < .05$; Table 1). Concerning the environmental benzene levels, the level in the police group was 0.15 mg/m³ whereas the level in the controls is beneath the sensitivity of the test.

DISCUSSION

Benzene is a common toxic volatile substance, found in many industrial processes in the present day [1]. It is classified as a carcinogen and can cause serious health problems. Immediate effects of high inhalation exposure can include headache, tiredness, nausea and dizziness, and unconsciousness may occur if exposure is very high. Recent research indicating that benzene exposure can result in chronic toxicity. The toxicity includes genotoxicity, neurotoxicity, and hematotoxicity.

International organizations such as Agency for Toxic Substances and Disease Registry (ATSDR) [6] have documented benzene toxicity and recommend the monitoring of benzene exposure for groups at risk. The California Environmental Protection Agency (CalEPA) has established a reference exposure level of less than 0.06 milligrams per cubic meter (0.06 mg/m³) for benzene [7]. At lifetime exposures increasingly greater than the reference exposure level, the potential for adverse health effects increases [7]. The work environment is, in many countries, strictly regulated with regard to the air

concentration of benzene. However, in exposure and risk-evaluation, the monitoring of benzene by peripheral biomarker has several advantages over technical assessment of exposure. For this purpose, the urine ttMA level is accepted as a useful monitoring tool for early diagnosis of dangerous exposure [8-9].

In Thailand, some previous studies on the urine ttMA levels in industrial workers have been documented for a few years [3-4]. Here, we provide data on this topic in non-industrial workers in the at-risk population. In our study, we found a high average level of urine ttMA among the policeman. Significantly higher than the controls can be observed, and we expected to find this difference theoretically. In addition, we detected high environmental benzene level in the police group whereas the level in the control group was very low. The results agree with the report of Crebelli et al. [10], which stated that the exposure to traffic fumes during working activities in traffic areas give a relatively greater contribution to personal exposure to benzene.

Comparing the average level in this study to the previous reports among the subjects living in the residents in the same area (0.05 ± 0.12 mg/gCr) [11], the significant higher ttMA level was detected in our police. However, comparing between the average ttMA level among the police to that of the Thai gas station attendant (4.00 ± 12.49 mg/gCr) in a previous report of Wiwanitkit et al. [4], lower values were observed in the police group. These results confirm the report of Priante et al. [12] that although traffic police exposure to benzene is higher than general residents it is much lower than that of other occupational categories such as fuel-pump distributors. Also, our results support the report of Crebelli et al. among the police in Rome that the exposure to traffic fumes during working activities may give a relatively greater contribution to general personal

exposure to benzene than indoor sources [13].

In this study, our controls showed level of ttMA beneath the sensitivity of the test and assumed as < 0.05 mg/gCr. This level is different from those reported among controls in our previous studies [3-4]. This may be due to the fact that we match the controls to the studied group and controlled the confounding factors including smoking, age, sex and diet, perhaps better than those previous studies [3-4]. The controls in our previous studies were not excluded for the environmental benzene exposure. In addition, our previous studies [3-4] compared industrial directly to non-industrial subjects while both controls and police in this study were non-industrial workers with difference in environmental exposure. The higher environmental benzene levels could be detected in our study.

Indeed, Leong and Laortanakul recently reported good correlation of blood benzene levels and ttMA with environmental benzene level in a recent study among the populations living in some traffic jam areas of Bangkok [14]. They reported the environmental benzene level ranging from 0.02 to 0.04 mg/m³ [14], lower than that of the police group in our study. In this study, we studied the police comparing to the control in the same area. Also, we can demonstrate higher ttMA and environmental benzene level among the police working in the road [14]. The high environmental benzene level among our police can imply the high benzene exposure. Of interest, this level is higher than the reference of CalEPA [7], therefore, the urgent corrective action for this finding is necessary. Indeed, the previous study of Verma et al. among Indian police also presented similar result [15]. Therefore, working in the air pollution in the urban area can be a health hazard for the police. Exposure to the benzene from automobile exhaust [16] can be an important occupa-

tional problem for these police. Of interest, although a protection device was distributed to these police, the high urine ttMA can be observed. In addition, none recalled a past health check-up for benzene exposure. The considerations for prevention of possibly exposure to benzene among the traffic police are recommended.

Some limitations of this study should be noted. Firstly, the sample size in this study is relatively small due to the nature of a pilot study. Secondly, although we tried our best to control the confounding factors there might be some cases disguising the answers to the questionnaire screening. Finally, the blood benzene levels for the subjects were not studied for its correlation to the ttMA, biomarker, in our study. We plan for a further larger study to cover those topics. Further studies focusing on the effect of benzene exposure on body systems such as hematotoxicity and genotoxicity are also recommended.

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